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Office of Research and Development

National Exposure Research Laboratory
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Athens, Georgia
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STANDARD OPERATING PROCEDURE

Title: Extraction of Perfluorinated Compounds (PFCs) from Sludge Samples
with Solid-Phase-Extraction or Ion-Pairing Cleanup

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SOP was Developed

☒ In-house

☐ Extramural

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**Extraction of Perfluorinated Compounds (PFCs) from Sludge Samples
with Solid-Phase-Extraction or Ion-Pairing Cleanup**

I. REAGENTS:

A. Polished 18MΩ Water

1. To polish water, i.e., purge of PFCs, use a glassware system dedicated to water polishing.
2. Pass 2L 18MΩ water through a 60cc "Oasis HLB" cartridge (use the same cartridge no more than 3 times).
3. Store polished 18MΩ water in dedicated 1L containers.

B. Polished Tetrabutylammonium (TBA) Mix (Ion Pairing Reagent)

1. Prepare 0.50 M Tetrabutylammonium Hydrogen Sulfate (TBAHS) in polished 18-MΩ water.
2. Prepare 0.25 M Na₂CO₃ in polished 18-MΩ water.
3. Add 2.0 parts Na₂CO₃ solution to 1.0 part TBAHS solution, mixing slowly to avoid spillage due to CO₂ generation.
4. Place a 500-mL Nalgene waste collection bottle in the reservoir of a Waters or comparable solid-phase extraction (SPE) vacuum system.
5. Mount a 35 cc HLB cartridge on the port above the Nalgene bottle.
6. Flush with 50 mL polished 18MΩ water and 50 mL methanol, HPLC grade.
7. Replace the waste Nalgene bottle with a methanol-washed Nalgene bottle; and discard the waste.
8. Pass the TBA Mix in part I.B.3 through the cartridge until desired volume has been polished; cap and label polished TBA mix.
9. Flush cartridge with 50 mL methanol (MeOH) per steps I.B.4 and I.B.6. Store in labeled Ziploc bag for further use in polishing this reagent mix only.

C. ¹³C₈-PFOA (M8C8) and ¹³C₄-PFOS (M4S8) Extraction Recovery Spike Solution

1. Prepare from Wellington Certified Stock Solutions in 60/40 (v/v) ACN/polished 18MΩ water to give a concentration of ~100 ng M8C8 and M4S8 per gram of solution as a mixture.

D. A Suite of Internal Matrix Standard Solution

1. Prepare from Wellington Certified Stock Solution in 60/40 (v/v) ACN/polished 18MΩ water to give a concentration of 0.05- 0.1 ng mass-labeled PFC standards per gram of solution; ¹³C₂-PFHxA, ¹³C₄-PFOA, ¹³C₅-PFNA, ¹³C₂-PFDA, ¹³C₂-PFUnDA, and ¹³C₂-PFDoDA

E. 2.0 M NaOH Solution and 2.0 M HCl Solution

1. Prepare from concentrated stock solutions using polished 18M Ω water.

II. SLUDGE SAMPLE EXTRACTION:**A. Prepare Bulk Sludge Sample and Dry Weight Measurement**

1. In hood, let the sludge/water sample settle overnight. At the end of sedimentation, decant the overlying water.
2. Weigh three ~0.5-2 gram aliquots of moist sludge into tared weighing boats; vacuum dry over Drierite for 18 hours and weigh again.
3. Repeat step II.A.2 as needed until constant weight is obtained. Calculate percent moisture of sludge.

B. Prepare Spiked Sludge Sample

1. Charge 0.5g-wet weight equivalent of sludge to pre-weighed (tube and cap) MeOH-washed, 16-mL polypropylene copolymer (PPCO) centrifuge tubes with size-18 PPCO caps. Re-weigh and record weight in data table.
2. Add 60 μ L of a mixture of M8C8 and M4S8 extraction recovery solution to provide a loading of no more than 10 ng M8C8 and M4S8 per gram of wet sludge. Vortex. Reweigh.
3. Add 0.25 mL of 2.0 M NaOH, vortex and sonicate for 30 min in a hot bath.
4. Let stand overnight for further reaction and equilibration.

C. Extract Spiked Sludge Sample

1. Neutralize the sample solution by adding 0.25 mL of 2.0 M HCl and vortex until homogeneous appearance.
2. Add 10 mL of 50:50/ACN:MeOH to a PPCO tube and sonicate for 30 min in a hot bath.
3. Place on an Eberbach Shaker® or equivalent and shake moderately for an hour.
4. Centrifuge in Sorvall® or equivalent at 17,500 rpm (~10,000 \times g) and 18 to 22 °C for 20 min.
5. Decant supernatant into a pre-weighed 40-mL glass vial.
6. Repeat step II.C.2 through II.C.5. Reweigh.
7. Transfer 2 mL sludge extract to a pre-weighed 12-mL glass vial. Reweigh.
8. Evaporate to dryness in SPE assembly, using nylon filters and 5 psi vacuum. Be sure not to bake the extract while drying it.

D. Two cleanup methods are available; both give good recoveries, but, in general, PFC peaks with solid-phase-extraction (SPE) cleanup have slightly better separation and shape than those from ion-pairing cleanup (IPCU).

1. SPE Cleanup method using Hydrophilic-Lipophilic-Balance (HLB) cartridge
 - i. Take 2 mL extract into 125-mL Nalgene bottle and dilute with 98mL polished 18M Ω water.
 - ii. Acidify a diluted extract with 0.15 mL glacial acetic acid (pH ~4).
 - iii. Position HLB cartridges in SPE assembly.

- iv. Condition cartridges by drawing through 5 mL MeOH.
- v. Equilibrate cartridges by drawing through 5 mL polished 18M Ω water.
- vi. Load a diluted sample (~100 mL). It is recommended to maintain flow rate at 1.0 – 1.5 mL/min for sample loading.
- vii. Wash cartridges with 5 mL of 25:75/MeOH:polished 18M Ω water (v/v).
- viii. Turn on vacuum to remove any residuals in cartridge and discard waste fluids.
- ix. Set up pre-weighed 12-mL vials for sample collection.
- x. Collect the eluates containing target PFCs by drawing through 5 mL MeOH. It is recommended to maintain flow rate at 1.0 – 1.5 mL/min for sample elution.
- xi. Evaporate and reconstitute with 1 mL 60:40/ACN:polished 18M Ω water containing a suite of matrix internal standards to give final mass-labeled PFC concentrations in the range of 0.05 to 0.1 ng/g. Reweigh.
- xii. Analyze by LC/MS/MS or other suitable method.

2. IPCU method

- i. Add 4 mL TBA Mix to dried extract from II.C.8. Vortex. Reweigh (*optional*).
- ii. Add 5 mL methyl-tert-butyl-ether (MTBE). Vortex for 30 sec., let rest and separate for about 30 min. and vortex for 30 sec. again. Reweigh (*optional*).
- iii. Freeze. Once water is frozen, transfer MTBE to tared 12-mL glass vial. Reweigh (*optional*).
- iv. Evaporate to dryness in SPE apparatus. Reweigh (*optional*).
- v. Reconstitute with 1 mL of 60:40/ACN:polished 18M Ω water containing a suite of matrix internal standards to give final mass-labeled PFC concentrations in the range of 0.05 to 0.1 ng/g. Reweigh.
- vi. Analyze by LC/MS/MS or other suitable method.

